PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, 5/10, C07K 14/50, 14/515

A2

(11) International Publication Number: **WO 00/05369**

(43) International Publication Date: 3 February 2000 (03.02.00)

(21) International Application Number: PCT/US99/12839

(22) International Filing Date: 20 July 1999 (20.07.99)

(30) Priority Data:

60/093,397	20 July 1998 (20.07.98)	US
09/150,684	10 September 1998 (10.09.98)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	60/093,397 (CIP)
Filed on	20 July 1998 (20.07.98)
US	09/150,684 (CIP)
Filed on	10 September 1998 (10.09.98)

(71) Applicant *(for all designated States except US)*: CURAGEN CORPORATION [US/US]; 11th floor, 555 Long Wharf Drive, New Haven, CT 06511 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SHIMKETS, Richard, A. [US/US]; 191 Leete Street, West Haven, CT 06516 (US).

(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P. C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: NUCLEOTIDE SEQUENCES AND AMINO ACID SEQUENCES OF SECRETED PROTEINS INVOLVED IN ANGIOGENESIS

(57) Abstract

Novel angiogenesis/anti–angiogenesis secreted proteins and the nucleic acid sequences which encode them are disclosed by the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

NUCLEOTIDE SEQUENCES AND AMINO ACID SEQUENCES OF SECRETED PROTEINS INVOLVED IN ANGIOGENESIS

FIELD OF THE INVENTION

The present invention provides novel amino acid sequences of potent angiogenesis/anti-angiogenesis secreted proteins and polynucleotide sequences encoding such proteins, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins, and derivatives, fragments and analogs thereof.

BACKGROUND OF THE INVENTION

Under normal physiological conditions, humans or animals undergo angiogenesis, i.e., generation of new blood vessels into a tissue or organ, only in restricted situations. During angiogenesis, endothelial cells react to stimulation with finely tuned signaling responses. The "endothelium" is a thin layer of flat epithelial cells that lines serous cavities, lymph vessels, and blood vessels. In normal physiological states such as embryonic growth and wound healing, neovascularization is controlled by a balance of stimulatory and inhibitory angiogenic factors. These controls may fail and result in formation of an extensive capillary network during the development of many diseases including ischemic heart disease, ischemic peripheral vascular disease, tumor growth and metastasis, reproduction, embryogenesis, wound healing, bone repair, rheumatoid arthritis, diabetic retinopathy and other diseases (for review, see e.g. Battegay, 1995).

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological disease states in which unregulated angiogenesis is present have been grouped together as angiogenic dependent or angiogenic associated diseases.

The balance of positive or negative angiogenesis regulators control the fate of vascular wall cells. They remain either in a state of vascular homeostasis, or they proceed to neovascularization, e.g., tumor growth and the switch to an angiogenic tumor phenotype correlates with increased secretion of angiogenic molecules such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and others. On the other hand, tumors also acquire a more angiogenic phenotype because inhibitors of angiogenesis are down-regulated during tumorigenesis (e.g. thrombospondin)(Dameron et al., 1994, Science 265:1582-1584).

Angiogenic and antiangiogenic (or angiostatic) molecules control the formation of new vessels via different mechanisms. Antiangiogenic molecules, or angiogenesis inhibitors (e.g. angiostatin, angiopoeitin-1 (Ang11), rat microvascular endothelial differentiation gene (MEDG), somatostatin, thrombospondin, platelet factor 4) can repress angiogenesis, and therefore, maintain vascular homeostasis (see, e.g. for review Bicknell, 1994, Ann. Oncol. 5 (suppl) 4:45-50).

Angiogenic molecules are capable of inducing the formation of new vessels and include, for example, but not for limitation, fibroblast growth factor (FGF), angiopoeitin 2 (Ang-2), erythroipoietin, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and others (for review, see e.g. Folkman & Shing, 1992, J. Biol. Chem. 267:10931-10934). FGF elicit its effects mainly via direct action on relevant endothelial cells via its endothelial receptor (e.g. Folkman & Shing, 1992, J. Biol. Chem. 267:10931-10934). FGF lacks a signal sequence for secretion.

Angiogenesis has been implicated in ischemic heart and ischemic vascular disease. In myocardial infarction new vessels penetrate the necrotic area and the surrounding schemic tissue. Neovascularizations, together with inflammatory cells, remove cellular debris and play a role in tissue repair and remodeling that results in myocardial scar formation. FGF-induced mycoardial infarction and neovascularization (e.g. Yanagisawa-Miwa et al., 1992, Science 257:1401-1403;

Harada et al., 1994, J. Clin. Invest. 94:623-630) show that angiogenesis contributes to the preservation of ischemic tissue and myocardial pump function in myocardial necrosis. This suggests a therapeutic use of angiogenic factors in clinical situations. Additional studies with FGF (Pu et al., 1993, Circulation 88:208-215) and VEGF (Takeshita et al., 1994, J. Clin. Invest 93:662-670) in peripheral ischemic vascular disease protected ischemic limbs. Similar to myocardial infarction, brain infarcts (strokes) are associated with angiogenesis (Chen et al., 1994, Stroke 25-1651-1657).

Likewise, angiogenesis has been implicated in various cancers. Angiogenesis is an essential component of the metastatic pathway (see, e.g. Zetter, 1998, Ann. Rev. Med. 49:407-427). These blood vessels provide the principal pathway by which tumor cells exit the primary tumor site and enter the circulation. Tumor angiogenesis is regulated by the production of angiogenic stimulators including members of the FGF and VEGF families (see, e.g. Fernig & Gallaher, 1994, Prog. Growth Factor Res. 5:353-377). Tumors may also activate angiogenic inhibitors such as angiostatin (U.S. patent 5639725, herein incorporated by reference) and endostatin that can modulate angiogenesis both at the primary site and at downstream sites of metastasis. The potential use of these and other natural and synthetic angiogenic inhibitors as anticancer drugs is currently under intense investigation (see, e.g. Zetter, 1998, Ann. Rev. Med. 49:407-427). Such agents may have reduced toxicity and be less likely to generate drug resistance than conventional cytotoxic drugs. Clinical trials are now underway to develop optimum treatment strategies for antiangiogenic agents.

Angiopoietin-1 (Ang-1) is an angiogenic factor that signals through the endothelial cell-specific Tie2 receptor tyrosine kinase. Like VEGF, Ang-1is essential for normal endothelial developmental processes in the mouse (Davis et al., 1996, Cell 87:). Furthermore, Ang-1 induces the formation of capillary sprouts (Koblizek et al., 1998, Curr. Biol. 8:529-532). The protein is expressed only on endothelial cells and early hemopoietic cells (e.g., see Suri et al., 1996, Cell 87:1171-1180).

Angiopoietin-2 (Ang-2) is a naturally occurring antagonist for Ang1 and Tie2 and can disrupt blood vessel formation in the mouse embryos (see, eg. Maisonpierre et al., 1997, Science 277:55-). Ang-2 is expressed only at sites of vascular remodeling.

In animal models some angiogenesis-dependent diseases can be controlled via induction or inhibition of new vessel formation. Treatment of diseases by modulation of angiogenesis are currently tested in clinical trials. Thus the manipulation of new vessel formation in angiogenesis-dependent conditions such as wound healing, inflammatory diseases, ischemic heart and peripheral vascular disease, myocardial infarction, diabetic retinopathy, and cancer is likely to create new therapeutic options.

Thus, angiogenesis is believed to play a significant role in the metastasis of a cancer and in the ischemic heart and ischemic vascular disease. If this angiogenic activity could be repressed or eliminated, then the tumor, although present, would not grow. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system. If this angiogenic activity could be stimulated or induced, ischemic tissues in the heart and brain and mycocardial necrosis could be prevented. In the disease state, stimulation or induction of angiogenesis could avert the damage. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

Novel angiogenic and antiangiogenic molecules are needed, both to model unwanted growth of blood vessels, especially into tumors, and for therapies directed to preventing such unwanted growth. In certain antiangiogenic embodiments, the compositions and methods of this invention are useful in inhibiting the activity of endogenous growth factors in premetastatic tumors and preventing the formation of the capillaries in the tumors thereby inhibiting the growth of the tumors. The composition, and antibodies specific to the composition, should also be able to modulate the formation of capillaries in other angiogenic processes, such as wound healing and reproduction. Finally, the composition and method for inhibiting angiogenesis should preferably be non-toxic and produce few side effects.

In certain angiogenic embodiments, the compositions and methods of this invention are useful in stimulating the growth of blood vessels, especially in mycocardial infarction and other heart diseases or brain infarcts (strokes). The
composition should be able to overcome the necrotic effects of ischemic tissue and thereby prevent the effects of heart diseases or strokes. Finally, the composition and method for stimulating angiogenesis should preferably be non-toxic and produce few side effects.

SUMMARY OF THE INVENTION

The present invention is directed to novel molecules, referred to herein as "angiopoeitin-3 (Ang-3)", "human microvascular endothelial differentiation gene 1 (hMEDG1)" and "heart specific growth factor-8b (FGF-8b)" polypeptides, as well as nucleic acid sequences encoding those molecules.

In certain preferred embodiments, the novel nucleic acid sequences of this invention is operatively linked to one or more expression control sequences. The invention also provides a host cell, including bacterial, plant, yeast, insect and mammalian cells, that produce the novel polypeptides, whether the cell is transformed with the nucleic acid sequences encoding those proteins, or whether the cell is transformed with regulatory sequences to activate or enhance production of these proteins from an endogenous nucleic acid sequence encoding same.

Processes are also provided for producing a protein, which comprise growing a culture of host cells producing such proteins (as described above) in a suitable culture medium, and purifying the protein from the culture. The protein produced according to such methods is also provided by the present invention. In preferred embodiments the protein comprises an angiopoeitin-3, hMEDG1 and FGF-8b amino acid sequence or fragments thereof, the protein being substantially free from other mammalian proteins. Such compositions may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also contemplated by the present invention. Methods are also contemplated for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

The proteins disclosed in this invention are likely to play a role in angiogenesis. Accordingly, the compositions and methods of this invention are useful in anti-cancer and heart disease therapies. Diagnostic, prognostic and screening kits are also contemplated.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the nucleotide sequence and deduced amino acid sequence of angiopoeitin-3 (Ang-3). The signal sequence is underlined.

FIG. 2 depicts the nucleotide sequence and deduced amino acid sequence of hMEDG1. The signal sequence is underlined.

FIG. 3 depicts the nucleotide sequence and deduced amino acid sequence of heart specific growth factor 8b (FGF-8b). Panel A shows the preliminarily determined sequence, with the signal sequence underlined. Panel B shows the confirmed sequence.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "direct angiogenic/antiangiogenic molecules" means a molecule that elicits an effect on angiogenesis/antiangiogenesis *in vivo* upon exogenous administration or overexpression, that has an effect on relevant endothelial cells *in vitro* that is compatible with angiogenesis/antiangiogenesis, and the role of the molecule has been established in a process or disease.

This invention further provides for novel angiopoeitin-3, hMEDG1, or FGF-8b proteins and genes encoding those proteins, as well as contemplating derivatives, homologs, active fragments and analogs, from various species, particularly vertebrates, and more particularly mammals. In a preferred embodiment, the foregoing proteins and genes are of human origin. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is also contemplated in the present invention. In other specific embodiments, the fragment, derivative or analog is functionally active, *i.e.*, capable of exhibiting one or more functional activity associated with wild type Ang3, hMEDG1, or FGF-8b protein. Such functional activities include, but are not limited to, the stimulation or inhibition of angiogenesis and related disorders and the treatment of heart disease and related disorders. Such functional activities include further, but are not limited to, antigenicity [ability to bind (or compete with Ang-3, hMEDG1, or FGF-8b for

binding) to an anti-Ang3, anti-hMEDG1, or anti-FGF-8b antibody, respectively], immunogenicity (ability to generate an antibody that binds to anti-IP-Ang3, anti-IP-hMEDG1, or anti-IP-heart specific growth factor, respectively), etc.

1. COMPLEMENTARY DNA (cDNA) SNYTHESIS AND CLONING

Messenger RNA (mRNA) was purified from total cellular RNA isolated from various human organs which were commercially-available from Clontech (e.g., Fetal brain, heart, kidney, fetal liver, liver, lung, skeletal muscle, pancreas and placenta) utilizing an Oligotex™ cDNA synthesis kit (QIAGEN, Inc.; Chatsworth, CA). The first-strand of the cDNA was prepared from 1.0 μg of poly(A)$^+$ RNA with 200 pmols of oligo(dT)25V (wherein V = A, C or G) using 400 units of Superscript II reverse transcriptase (BRL; Grand Island NY). Following the addition of 10 units of *E. coli* DNA ligase, 40 units of *E. coli* DNA polymerase, and 3.5 units of *E. coli* RNase H (all supplied by BRL; Grand Island, NY), second-strand synthesis was performed at 16°C for 2 hours. Five units of T_4 DNA polymerase was then added, and incubation was continued for an additional 5 minutes at 16°C. The reaction was then treated with 5 units of arctic shrimp alkaline phosphatase (U.S. Biochemicals; Cleveland OH) at 37°C for 30 minutes, and the cDNA was purified by standard phenol/chloroform (50:50 v/v) extraction. The yield of cDNA was estimated using fluorometry with the Picogreen™ Labeling System (Molecular Probes; Eugene, OR).

Following synthesis, the double-stranded cDNA was digested with various restriction enzymes, ligated to linkers compatible with the over-hanging termini generated by the restriction digestion. The restriction fragments were amplified utilizing 30 cycles of polymerase chain reaction (PCR) by the addition of the following reagents: 2 μl 10 mM dNTP; 5 μl 10X TB buffer (500 mM Tris, 160 mM $(NH4)_2SO_4$,; 20 mM $MgCl_2$, pH 9.15); 0.25 μl Klentaq (Clontech Advantage) : PFU (Stratagene; La Jolla CA) in a 16:1 v/v ratio; 32.75 μl ddH2O. The amplification products were then ligated into the TA™ cloning vector (Invitrogen). Individual clones were subjected to dye-primer, double-stranded DNA sequencing utilizing PCR products which were derived from amplification using vector-specific primers, which flanked the insertion, site as templates. Sequencing was performed using a standard chemistry methdology on ABI Model 377 sequencers (Molecular Dynamics).

2. ISOLATED PROTEINS AND POLYNUCLEOTIDES, AND DERIVATIVES AND ANALOGS

The sequence of a polynucleotide encoding an Ang-3 protein of the present invention is set forth in Figure 1, with the coding region extending from nucleotides **352 to 1824**. This polynucleotide has been named "Angiopoeitin-3 (Ang-3)". The amino acid sequence of the protein encoded by Ang-3 is also shown in Figure 1. Ang-3 was isolated from a human heart library, cloned into a vector and sequenced by methods known in the art.

The nucleic acid sequence in Figure 1 encodes a novel protein, Ang-3. Sequence homology algorithms, e.g., BLASTN/BLASTX or FASTA searches, revealed no exact sequence matches. A BLASTX search revealed 59 % homology between Ang-3 (in the approximate region of nucleotides 1067 and 1833 of Figure 1), and human angiopoietin proteins (including human Ang1 [GenBank Accession Number U83508] and human Ang-2 [GenBank Accession Number AF004327]. A BLASTP search also revealed 63 % homology between Ang-3 (in the approximate region of amino acids 269 to 491 of Figure 1), and human angiopoietin proteins (including human angiopoetin-like protein (CDT6 gene) [GenBank Accession Number Y16132]). Furthermore, a BLASTP search revealed 51 % homology between Ang-3 (in the approximate region of amino acids 8 to 491 of Figure 1), and mouse angiopoietin-1 protein [GenBank Accession Number U83509]). Finally, a BLASTP search revealed 61 % homology between Ang-3 (in the approximate region of amino acids 277 to 491 of Figure 1) and fibrinogen-like proteins (including fibrinogen-like protein 1 precursor [TREMBL Accession Number Q08830]), fibrinogen-related protein HFREP-1 precursor [PIR Accession Number JN0596] and fibrinogen-like protein [TREMBLAccession Number Q143114]).

Analysis of the full-length Ang-3 sequence revealed that this clone contains a signal sequence. Ang-2 has a secretion signal peptide. Ang-1 and Ang-2 are 60 % identical. Furthermore, Ang-3 is homologous in the carboxy-terminal fibrinogen-like domain to Ang-1 and Ang-2 (Maisonpierre et al., 1997, Science 277:55-60). Based upon these homologies, Ang-3 and these homologous proteins are expected to share at least some activities.

Accordingly, in one embodiment, the present invention provides an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence shown in Figure 1 from nucleotide 352 to nucleotide 1824, or nucleotide 415 to nucleotide 1824;

(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 1 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 1, from nucleotide 352 to nucleotide 1824 (i.e., including the signal sequence) or from nucleotide 415 to nucleotide 1824 (the mature peptide), herein called angiopoetin-3;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 1 having biological activity;

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

The sequence of a polynucleotide encoding another protein of the present invention is set forth in Figure 2, with the coding region extending from nucleotides 190 to 858. This polynucleotide has been named "Microvascular Endothelial Differentiation Gene" (hMEDG1). The amino acid sequence of the protein encoded by the endothelial differentiation gene (hMEDG1) is set forth in Figure 2. HMEDG1 was isolated from a human heart library, cloned into a vector and sequenced by methods known in the art.

The nucleic acid sequence in Figure 2 encodes a novel protein, hMEDG1. Sequence homology algorithms, e.g., BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. A BLASTX search revealed 84 % homology between the hMEDG1 protein (in the approximate region of nucleotides 56-1013 of Figure 2 and rat microvascular endothelial differentiation gene 1 (GenBank Accession Number X98993). BLASTP search revealed 94 % homology between the hMEDG1 protein (in the approximate region of amino acids 51-223 of Figure 2) and rat microvascular endothelial differentiation gene 1 (SPTREMBL-Acc. No. P97554). Analysis of the protein sequences revealed that the N-terminus of hMEDG1 contains 50 amino acids that were not reported in the rat MEDG1 protein. These 50 amino acids comprise a signal sequence; therefore hMEDG1 does encode a secreted factor. Based upon these homologies, hMEDG1 and these homologous proteins are expected to share at least some activities.

Accordingly, in this embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence shown in Figure 2 from nucleotide 51 to nucleotide 858, or from nucleotide 259 to nucleotide 858;

(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 2 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 2, from nucleotide 51 to nucleotide 858 (i.e., including the signal sequence) or nucleotide 259 to nucleotide 858 (the mature peptide), herein called human microvascular endothelial differention gene 1;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 2 having biological activity;

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

The sequence of a polynucleotide encoding another protein of the present invention is set forth in Figure 3, with the coding region extending from nucleotides 39 to 618 (Panel A) and 39 to 659 (Panel B). This polynucleotide has been identified as "human heart specific fibroblast growth factor 8b (FGF-8b)" The amino acid sequence of the protein encoded by FGF-8b is set forth in Figure 3. The human FGF-8b was isolated from a human heart library using a trap which selects for nucleotides encoding secreted proteins; therefore, FGF-8b encodes a secreted factor.

The nucleic acid sequence in Figure 3 encodes a novel protein, FGF-8b. Panel A shows a preliminarily determined sequence. Panel B shows a confirmed sequence. We prefer the sequence in Panel B. Sequence homology algorithms, e.g., BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. A BLASTP search revealed about 80 % homology between the FGF-8b (particularly in the approximate region of amino acids 1-181 of Figure 3) and various human growth factors including fibroblast growth factor 8, androgen-induced growth factor, keratinocyte growth factor, heparin-binding growth factor-1, and beta-endothelial cell growth factor (including without limitation those assigned accession numbers G2660747, P55075, P36363, P10935, E68414). BLASTX searches confirmed these results. Based upon these homologies, FGF-8b and these homologous proteins are expected to share at least some activities.

Accordingly, in another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence shown in Figure 3 from nucleotide 39 to nucleotide 618, or from nucleotide 120 to nucleotide 618;

(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 3 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 3, from nucleotide 39 to nucleotide 618 (i.e., including the signal sequence) or nucleotide 120 to nucleotide 618 (the mature peptide), herein called heart specific growth factor 8b as initially determined;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 3 having biological activity;

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

Accordingly, in another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence shown in Figure 3B from nucleotide 39 to nucleotide 659, or from nucleotide 120 to nucleotide 618;

(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 3 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 3, from nucleotide 39 to nucleotide 659 (i.e., including the signal sequence) or nucleotide 120 to nucleotide 659 (the mature peptide), herein called heart specific growth factor 8b;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 3 having biological activity;

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

The tissue distribution of Ang-3, hMEDG1 and FGF-8b is shown in Table 1:

Table 1

	Heart	Lung	Brain	Kidney	Testis	Liver	Muscle	Pancreas	Bone
Ang-3	+++	+++	++	++	++	++	++	++	-
hMEDG1	+	-	-	-	-	-	-	-	-
FGF-8b	+++	++	+	-	-	-	+	-	-

+++ = strong expression
++ = moderate expression
+ = weak expression
- = expression not detected

Nucleic acids encoding Ang3, hMEDG1, or FGF-8b can be obtained by any method known in the art, *e.g*, by PCR amplification using synthetic primers that hybridize to the 3' and 5' ends of the sequence and/or by cloning from a cDNA or genomic library using an oligonucleotide specific for the gene sequence, *e.g.*, as described *infra*.

Homologs, *e.g.*, of nucleic acids encoding Ang3, hMEDG1, or FGF-8b of species other than human, or other related sequences, *e.g.*, paralogs, can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning, *e.g.*, as described *infra*, for Ang3, hMEDG1, or FGF-8b nucleotide sequences.

Polynucleotides hybridizing to the polynucleotides of the present invention under stringent conditions and highly stringent conditions are also part of the present invention. As used herein, "highly stringent conditions" include, for example, at least about 0.2 times SSC at 65 degree C; and "stringent conditions" include, for example, hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37 C, and a wash in 0.1X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 60-65 C. Preferred high stringency conditions are hybridization in 4X SSC, 5X Denhardt's (5 g Ficoll, 5 g polyvinylpyrrolidone, 5 g bovine serum albumin in 500 ml of water), 0.1 mg/ml boiled salmon sperm DNA, and 25 mM Na phosphate at 65 C, and a wash in 0.1X SSC, 0.1% SDS at 65 C. Allelic variants of the polynucleotides of the present invention are also encompassed by the invention.

3. EXPRESSION OF RECOMBINANT PROTEINS

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 ells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, Candida, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, Calif., U.S.A. (the MaxBac.R™. kit), and such methods are well known in the art, as described in Summers and

Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

4. BIOLOGICAL ACTIVITY OF PROTEIN FRAGMENTS

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein-IgM fusion would generate a decavalent form of the protein of the invention.

5. PURIFICATION OF THE PROTEIN

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl R ™. or Cibacrom blue 3GA Sepharose R™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and InVitrogen, respectively.

The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

6. MODIFICATION

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Mutagenic

techniques for such replacement. insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Pat. No. 4,518,584, incorporated by reference).

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

7. USES AND BIOLOGICAL ACTIVITY

The polynucleotides of the present invention and the proteins encoded thereby are expected to exhibit one or more of the uses or biological activities identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

The biological activity of the proteins of this invention can be assayed by any suitable method known in the art. The angiogenic/antiangiogenic potential can be characterized in angiogenesis assays *in vivo* such as the chick chorionic allantoic membrane (CAM) assay or different cornea micropocket assays (Klagsbrun & Folkman, 1990, In: Sporn & Roberts (eds). Peptide growth factors and their receptors II, pp. 549-574). An a *in vivo* angiogenesis assay is described in, eg., U.S. Pat. No. 5,382,514, incorporated by reference), and a mouse model of hindlimb ischemia was described by Couffinhal et al., 1998, Am. J. Pathol. 152:1667-1679). Direct effects of angiogenic molecules on vascular wall cells can be assayed in *in vitro* assays. These assays facilitate the study of endothelial functions that are essential for new blood vessel formation. Most *in vitro* models of angiogenesis use extracellular matrix substrata containing growth-regulatory molecules (Vukicevic et al., 1992, Exp. Cell. Res. 202:1-8). Furthermore, most assays cell culture assay for angiopoietin to test the formation of capillary sprouts (see, eg. Koblizek et al., 1998, Curr. Biol. 8:529-532).Most assays require exogenous stimuli such as phorbol esters or angiogenic molecules to induce the formation of endothelial cords and tubes. Assays for angiogenic/antiangiogenic activity include methods for inhibition of angiogenesis (see, for example, but not limited to, U.S. Pat. No. 5733876, 5639725, 5712291, 5698586,

5753230, 5733876, 5766591, 5434185, 5721226,5629340 5593990, 5629327, 5744492, 5646136, 5610166, 5574026, 5567693, 5563130, each herein fully incorporated by reference).

8. ANGIOGENIC STIMULATION/INHIBITION ACTIVITY

A protein of the present invention may exhibit angiogenic (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity. Many protein factors discovered to date, including all known angiopoeitins and growth factors, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of angiogenic activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10,B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1,123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

Methods of diagnosis, prognosis, and screening for diseases and disorders associated with aberrant levels of an Ang3, hMEDG1, or heart specific growth factor, are contemplated. The invention also contemplates methods of treating or preventing diseases or disorders associated with aberrant levels of an Ang3, hMEDG1, or heart specific growth factor, or aberrant levels of activity of one or more of the components of the complex, comprising administration of the Ang3, hMEDG1, or heart specific growth factor.

Methods of assaying an Ang3, hMEDG1, or FGF-8b, for activity as therapeutics or diagnostics as well as methods of screening modulators (*i.e.*, inhibitors, agonists and antagonists) are also contemplated.

9. ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition may further contain other agents which either

enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular Ang3, hMEDG1, or FGF-8b to minimize side effects of the Ang3, hMEDG1, or FGF-8b agent.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally-acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of

the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing Ang3, hMEDG1, or heart specific growth factor. When co-administered with one or more Ang3, hMEDG1, or FGF-8b, protein of the present invention may be administered either simultaneously with the Ang3, hMEDG1, or FGF-8b, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with Ang3, hMEDG1, or FGF-8b.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further.

10. ANTIBODY GENERATION

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R. P. Merrifield, J. Amer. Chem. Soc. 85, 2149-2154 (1963); J. L. Krstenansky, et al., FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous

cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

11. GENE THERAPY

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or ex vivo into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

WE CLAIM:

1. An isolated polynucleotide selected from the group consisting of:
(a) a polynucleotide comprising the nucleotide sequence shown in Figure 1 from nucleotide 352 to nucleotide 1824, or nucleotide 415 to nucleotide 1824;
(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 1 encoding a protein having biological activity;
(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 1, from nucleotide 352 to nucleotide 1824 (i.e., including the signal sequence) or from nucleotide 415 to nucleotide 1824 (the mature peptide), herein called angiopoetin-3;
(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 1 having biological activity;
(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

2. The polynucleotide of claim 1 wherein said polynucleotide is operably linked to an expression control sequence.

3. A host cell that produces the protein shown in Figure 1, such cell produced by having its genome augmented with the polynucleotide of claim 2 or with a regulatory sequence that induces or enhances expression of an endogenous polynucleotide according to claim 1.

4. The host cell of claim 3, wherein said cell is a mammalian cell.

5. A process for producing a protein, which comprises: (a) growing a culture of the host cell of claim 3 in a suitable culture medium; and
(b) purifying the protein from the culture.

6. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence of Figure 1 from nucleotide 415 to nucleotide 1824.

7. A protein comprising the amino acid sequence of Figure 1, encoded by nucleotide 352 to nucleotide 1824 or nucleotide 415 to nucleotide 1824, and functional analogs or derivatives thereof.

8. An isolated polynucleotide selected from the group consisting of:
(a) a polynucleotide comprising the nucleotide sequence shown in Figure 2 from nucleotide 51 to nucleotide 858, or from nucleotide 259 to nucleotide 858;
(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 2 encoding a protein having biological activity;
(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 2, from nucleotide 51 to nucleotide 858 (i.e., including the signal sequence) or nucleotide 259 to nucleotide 858 (the mature peptide), herein called human microvascular endothelial differention gene 1;
(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 2 having biological activity;
(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

9. The polynucleotide of claim 8 wherein said polynucleotide is operably linked to an expression control sequence.

10. A host cell that produces the protein shown in Figure 2, such cell produced by having its genome augmented with the polynucleotide of claim 9 or with a regulatory sequence that induces or enhances expression of an endogenous polynucleotide according to claim 8.

11. The host cell of claim 10, wherein said cell is a mammalian cell.

12. A process for producing a protein, which comprises: (a) growing a culture of the host cell of claim 10 in a suitable culture medium; and
(b) purifying the protein from the culture.

13. The polynucleotide of claim 8 wherein said polynucleotide comprises the nucleotide sequence of Figure 2 from nucleotide 259 to nucleotide 858.

14. A protein comprising the amino acid sequence of Figure 2 encoded by nucleotide 51 to nucleotide 858, or from nucleotide 259 to nucleotide 858.

15. An isolated polynucleotide selected from the group consisting of:
(a) a polynucleotide comprising the nucleotide sequence shown in Figure 3A from nucleotide 39 to nucleotide 618, or from nucleotide 120 to nucleotide 618;
(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 3A encoding a protein having biological activity;
(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 3A, from nucleotide 39 to nucleotide 618 (i.e., including the signal sequence) or nucleotide 120 to nucleotide 618 (the mature peptide);
(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 3A having biological activity;
(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

16. The polynucleotide of claim 15 wherein said polynucleotide is operably linked to an expression control sequence.

17. A host cell that produces the protein shown in Figure 3A, such cell produced by having its genome augmented with the polynucleotide of claim 16 or with a regulatory sequence that induces or enhances expression of an endogenous polynucleotide according to claim 15.

18. The host cell of claim 17, wherein said cell is a mammalian cell.

19. A process for producing a protein, which comprises:
(a) growing a culture of the host cell of claim 17 in a suitable culture medium; and
(b) purifying the protein from the culture.

20. The polynucleotide of claim 15 wherein said polynucleotide comprises the nucleotide sequence of Figure 3A from nucleotide 120 to nucleotide 618.

21. A protein comprising the amino acid sequence of Figure 3A encoded by nucleotide 39 to nucleotide 618 or nucleotide 120 to nucleotide 618.

22. An isolated polynucleotide selected from the group consisting of:
(a) a polynucleotide comprising the nucleotide sequence shown in Figure 3B from nucleotide 39 to nucleotide 659, or from nucleotide 120 to nucleotide 659;
(b) a polynucleotide comprising a fragment of the nucleotide sequence shown in Figure 3B encoding a protein having biological activity;
(c) a polynucleotide encoding a protein comprising the amino acid sequence shown in Figure 3B, from nucleotide 39 to nucleotide 659 (i.e., including the signal sequence) or nucleotide 120 to nucleotide 659 (the mature peptide);
(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence shown in Figure 3B having biological activity;
(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

23. The polynucleotide of claim 22 wherein said polynucleotide is operably linked to an expression control sequence.

24. A host cell that produces the protein shown in Figure 3B, such cell produced by having its genome augmented with the polynucleotide of claim 23 or with a regulatory sequence that induces or enhances expression of an endogenous polynucleotide according to claim 22.

25. The host cell of claim 24, wherein said cell is a mammalian cell.

26. A process for producing a protein, which comprises:
(a) growing a culture of the host cell of claim 24 in a suitable culture medium; and
(b) purifying the protein from the culture.

27. The polynucleotide of claim 22 wherein said polynucleotide comprises the nucleotide sequence of Figure 3B from nucleotide 120 to nucleotide 659.

28. A protein comprising the amino acid sequence of Figure 3B encoded by nucleotide 39 to nucleotide 618 or nucleotide 120 to nucleotide 659.

Angiopoeitin-3

```
   1
     ATCTGGGTCAGCTGCAGCTGGTTACTGCATTTCTCCATGTGGCAGACAGAGCAAAGCCACAACGCTTTCTCTGCTGGATT
  81
     AAAGACGGCCCACAGACCAGAACTTCCACTATACTACTTAAAATTACATAGGTGGCTTGTCAAATTCAATTGATTAGTAT
 161
     TGTAAAAGGAAAAAGAAGTTCCTTCTTACAGCTTGGATTCAACGGTCCAAAACAAAAATGCAGCTGCCATTAAAGTCACA
 241
     GATGAACAAACTTCTACACTGATTTTTAAAATCAAGAATAAGGGCAGCAAGTTTCTGGATTCACTGAATCAACAGACACA
 321
     AAAAGACATCATTTTACAACCTCATTTCAAAATGAAGACTTTTACCTGGACCCTAGGTGTGCTATTCTTCCTACTAGTGG
                                      MetLysThrPheThrTrpThrLeuGlyValLeuPhePheLeuLeuValA
 401
     ACACTGGACATTGCAGAGGTGGACAATTCAAAATTAAAAAAATAAACCAGAGAAGATACCCTCGTGCCACAGATGGTAAA
     spThrGlyHisCysArgGlyGlyGlnPheLysIleLysLysIleAsnGlnArgArgTyrProArgAlaThrAspGlyLys
 481
     GAGGAAGCAAAGAAATGTGCATACACATTCCTGGTACCTGACCAAAGAATAACAGGGCCAATCTGTGTCAACACCAAGGG
     GluGluAlaLysLysCysAlaTyrThrPheLeuValProAspGlnArgIleThrGlyProIleCysValAsnThrLysGl
 561
     GCAAGATGCAAGTACCATTAAAGACATGATCACCAGGATGGACCTTGAAAACCTGAAGGATGTGCTCTCCAGGCAGAAGC
     yGlnAspAlaSerThrIleLysAspMetIleThrArgMetAspLeuGluAsnLeuLysAspValLeuSerArgGlnLysA
 641
     GGGAGATAGATGTTCTGCAACTGGTGGTGGATGTAGATGGAAACATTGTGAATGAGGTAAAGCTGCTGAGAAAGGAAAGC
     rgGluIleAspValLeuGlnLeuValValAspValAspGlyAsnIleValAsnGluValLysLeuLeuArgLysGluSer
 721
     CGTAACATGAACTCTCGTGTTACTCAACTCTATATGCAATTATTACATGAGATTATCCGTAAGAGGGATAATTCACTTGA
     ArgAsnMetAsnSerArgValThrGlnLeuTyrMetGlnLeuLeuHisGluIleIleArgLysArgAspAsnSerLeuGl
 801
     ACTTTCCCAACTGGAAAACAAAATCCTCAATGTCACCACAGAAATGTTGAAGATGGCAACAAGATACAGGGAACTAGAGG
     uLeuSerGlnLeuGluAsnLysIleLeuAsnValThrThrGluMetLeuLysMetAlaThrArgTyrArgGluLeuGluV
 881
     TGAAATACGCTTCCTTGACTGATCTTGTCAATAACCAATCTGTGATGATCACTTTGTTGGAAGAACAGTGCTTGAGGATA
     alLysTyrAlaSerLeuThrAspLeuValAsnAsnGlnSerValMetIleThrLeuLeuGluGluGlnCysLeuArgIle
 961
     TTTTCCCGACAAGACACCCATGTGTCTCCCCCACTTGTCCAGGTGGTGCCACAACATATTCCTAACAGCCAACAGTATAC
     PheSerArgGlnAspThrHisValSerProProLeuValGlnValValProGlnHisIleProAsnSerGlnGlnTyrTh
1041
     TCCTGGTCTGCTGGGAGGTAACGAGATTCAGAGGGATCCAGGTTATCCCAGAGATTTAATGCCACCACCTGATCTGGCAA
     rProGlyLeuLeuGlyGlyAsnGluIleGlnArgAspProGlyTyrProArgAspLeuMetProProProAspLeuAlaT
1121
     CTTCTCCCACCAAAAGCCCTTTCAAGATACCACCGGTAACTTTCATCAATGAAGGACCATTCAAAGACTGTCAGCAAGCA
     hrSerProThrLysSerProPheLysIleProProValThrPheIleAsnGluGlyProPheLysAspCysGlnGlnAla
```

Fig. 1

```
1201
    AAAGAAGCTGGGCATTCGGTCAGTGGGATTTATATGATTAAACCTGAAAACAGCAATGGACCAATGCAGTTATGGTGTGA
    LysGluAlaGlyHisSerValSerGlyIleTyrMetIleLysProGluAsnSerAsnGlyProMetGlnLeuTrpCysGl
1281
    AAACAGTTTGGACCCTGGGGGTTGGACTGTTATTCAGAAAAGAACAGACGGCTCTGTCAACTTCTTCAGAAATTGGGAAA
    uAsnSerLeuAspProGlyGlyTrpThrValIleGlnLysArgThrAspGlySerValAsnPhePheArgAsnTrpGluA
1361
    ATTATAAGAAAGGGTTTGGAAACATTGACGGAGAATACTGGCTTGGACTGGAAAATATCTATATGCTTAGCAATCAAGAT
    snTyrLysLysGlyPheGlyAsnIleAspGlyGluTyrTrpLeuGlyLeuGluAsnIleTyrMetLeuSerAsnGlnAsp
1441
    AATTACAAGTTATTGATTGAATTAGAAGACTGGAGTGATAAAAAAGTCTATGCAGAATACAGCAGCTTTCGTCTGGAACC
    AsnTyrLysLeuLeuIleGluLeuGluAspTrpSerAspLysLysValTyrAlaGluTyrSerSerPheArgLeuGluPr
1521
    TGAAAGTGAATTCTATAGACTGCGCCTGGGAACTTACCAGGGAAATGCAGGGGATTCTATGATGTGGCATAATGGTAAAC
    oGluSerGluPheTyrArgLeuArgLeuGlyThrTyrGlnGlyAsnAlaGlyAspSerMetMetTrpHisAsnGlyLysG
1601
    AATTCACCACACTGGACAGAGATAAAGATATGTATGCAGGAAACTGCGCCCACTTTCATAAAGGAGGCTGGTGGTACAAT
    lnPheThrThrLeuAspArgAspLysAspMetTyrAlaGlyAsnCysAlaHisPheHisLysGlyGlyTrpTrpTyrAsn
1681
    GCCTGTGCACATTCTAGCCTAAATGGAGTATGGTACAGAGGAGGCCATTACAGAAGCAAGCACCAAGATGGAATTTTCTG
    AlaCysAlaHisSerSerLeuAsnGlyValTrpTyrArgGlyGlyHisTyrArgSerLysHisGlnAspGlyIlePheTr
1761
    GGCCGAATACAGAGGCGGGTCATACTCCTTAAGAGCAGTTCAGATGATGATCAAGCCTATTGACTGAAGAGAGACACTCG
    pAlaGluTyrArgGlyGlySerTyrSerLeuArgAlaValGlnMetMetIleLysProIleAsp
1841
    CCAATTTAAATGACACAGAACTTTGTACTTTTCAGCTCTTAAAAATGTAAATGTTACATGTATATTACTTGGCACAATTT
1921
    ATTTCTACACATAAAGTTTTTAAAATGAATTTTACCGTAACTATAAAAGGGAACCTATAAATGTAGTTTCATCTGTCGTC
2001
    AATTACTGCAGAAAATTATGTGTATCCACAACCTAGTTATTTTAAAAATTATGTTGACTAAATACAAAGTTTGGTTTCTA
2081
    AAATGTAAATATTTGCCACAATGTAAAGCAAATCTTAGCTATATTTTAAATCATAAATAACATGTTCAAGATACTTAACA
2161
    ATTTATTTAAAATCTAAGATTGCTCTAACGTCTAGTGAAAAAAATATTTTTAAAATTTCAGCCAAATGATGCATTTTATT
2241
    TATAAAAATACAGACAGAAAATTAGGGAGAAACCTCTAGTTTTGCCAATAGAAAATGCTTCTTCCATTGAATAAAAGTTA
2321
    TTTCAAATCCAAAAAAAAAAAAAAAAAAAAAAA
```

Fig. 1 (cont.)

hMEDG1

```
1
CGCCCGGGCAGTGCCCGGGGAAGGAGGAGCGCTAGGTCGGTGTACGACCGAGATTAGGGTGCGTGCCAGCTCCGGGAGGC
81
CGCGGTGAGGGGCCGGGCCCAAGCTGCCGACCCGAGCCGATCGTCAGGGTCGCCAGCGCCTCAGCTCTGTGGAGGAGCAG
161
CAGTAGTCGGAGGGTGCAGGATATTAGAAATGGCTACTCCCCAGTCAATTTTCATCTTTGCAATCTGCATTTTAATGATA
                                MetAlaThrProGlnSerIlePheIlePheAlaIleCysIleLeuMetIle
241
ACAGAATTAATTCTGGCCTCAAAAAGCTACTATGATATCTTAGGTGTGCCAAAATCGGCATCAGAGCGCCAAATCAAGAA
ThrGluLeuIleLeuAlaSerLysSerTyrTyrAspIleLeuGlyValProLysSerAlaSerGluArgGlnIleLysLy
321
GGCCTTTCACAAGTTGGCCATGAAGTACCACCCTGACAAAAATAAGAGCCCGGATGCTGAAGCAAAATTCAGAGAGATTG
sAlaPheHisLysLeuAlaMetLysTyrHisProAspLysAsnLysSerProAspAlaGluAlaLysPheArgGluIleA
401
CAGAAGCATATGAAACACTCTCAGATGCTAATAGACGAAAAGAGTATGATACACTTGGACACAGTGCTTTTACTAGTGGT
laGluAlaTyrGluThrLeuSerAspAlaAsnArgArgLysGluTyrAspThrLeuGlyHisSerAlaPheThrSerGly
481
AAAGGACAAAGAGGTAGTGGAAGTTCTTTTGAGCAGTCATTTAACTTCAATTTTGATGACTTATTTAAAGACTTTGGCTT
LysGlyGlnArgGlySerGlySerSerPheGluGlnSerPheAsnPheAsnPheAspAspLeuPheLysAspPheGlyPh
561
TTTTGGTCAAAACCAAAACACTGGATCCAAGAAGCGTTTTGAAAATCATTTCCAGACACGCCAGGATGGTGGTTCCAGTA
ePheGlyGlnAsnGlnAsnThrGlySerLysLysArgPheGluAsnHisPheGlnThrArgGlnAspGlyGlySerSerA
641
GACAAAGGCATCATTTCCAAGAATTTTCTTTTGGAGGTGGATTATTTGATGACATGTTTGAAGATATGGAGAAAATGTTT
rgGlnArgHisHisPheGlnGluPheSerPheGlyGlyGlyLeuPheAspAspMetPheGluAspMetGluLysMetPhe
721
TCTTTTAGTGGTTTTGACTCTACCAATCAGCATACAGTACAGACTGAAAATAGATTTCATGGATCTAGCAAGCACTGCAG
SerPheSerGlyPheAspSerThrAsnGlnHisThrValGlnThrGluAsnArgPheHisGlySerSerLysHisCysAr
801
GACTGTCACTCAACGAAGAGGAAATATGGTTACTACATACACTGACTGTTCAGGACAGTAGTTCTTATTCTATTCTCACT
gThrValThrGlnArgArgGlyAsnMetValThrThrTyrThrAspCysSerGlyGln
881
AAATCCAACTGGTTGACTCTTCCTCATTATCTTTGATGCTAAACAATTTTCTGTGAACTATTTTGACAAGTGCATGATTT
961
CACTTTAAACAATTTGATATAGCTATTAAATATATTTAAGGGTTTTTTTTTTTG
```

Fig. 2

FGF-8b

```
1
GGCTGGGCTAGGAGCCGCCGCCTCCCTCCCGCCCAGCGATGTATTCAGCGCCCTCCGCCTGCACTTGCCTGTGTTTACAC
                                  MetTyrSerAlaProSerAlaCysThrCysLeuCysLeuHis
81
TTCCTGCTGCTGTGCTTCCAGGTACAGGTGCTGGTTGCCGAGGAGAACGTGGACTTCCGCATCCACGTGGAGAACCAGAC
PheLeuLeuLeuCysPheGlnValGlnValLeuValAlaGluGluAsnValAspPheArgIleHisValGluAsnGlnTh
161
GCGGGCTCGGGACGATGTGAGCCGTAAGCAGCTGCGGCTGTACCAGCTCTACAGCCGGACCAGTGGGAAACACATCCAGG
rArgAlaArgAspAspValSerArgLysGlnLeuArgLeuTyrGlnLeuTyrSerArgThrSerGlyLysHisIleGlnV
241
TCCTGGGCCGCAGGATCAGTGCCCGCGGCGAGGATGGGGACAAGTATGCCCAGCTCCTAGTGGAGACAGACACCTTCGGT
alLeuGlyArgArgIleSerAlaArgGlyGluAspGlyAspLysTyrAlaGlnLeuLeuValGluThrAspThrPheGly
321
AGTCAAGTCCGGATCAAGGGCAAGGAGACGGAATTCTACCTGTGCATGAACCGCAAAGGCAAGCTCGTGGGGAAGCCCGA
SerGlnValArgIleLysGlyLysGluThrGluPheTyrLeuCysMetAsnArgLysGlyLysLeuValGlyLysProAs
401
TGGCACCAGCAAGGAGTGTGTGTTCATCGAGAAGGTTCTGGAGAACAACTACACGGCCCTGATGTCGGCTAAGTACTCCG
pGlyThrSerLysGluCysValPheIleGluLysValLeuGluAsnAsnTyrThrAlaLeuMetSerAlaLysTyrSerG
481
GCTGGTACGTGGGCTTTACCAAGAAGGGGCGGCCGCGGAAGGGCCCCAAGACCCGGGAGAACCAGCAGGACGTGCATTTC
lyTrpTyrValGlyPheThrLysLysGlyArgProArgLysGlyProLysThrArgGluAsnGlnGlnAspValHisPhe
561
ATTGAAGCGCTACCCCAAGGGGCAACCCGGAGCTTTAGAAGCCCTTCAAGTACACGACNGTGACCAAGAAGTCCCGTCCG
IleGluAlaLeuProGlnGlyAlaThrArgSerPheArgSerProSerSerThrArg---
641
GATCCGGCCCACACACCCTGCCTAAGGGCAACCCGCCGCGGGGCCCCT
```

Fig. 3A

FGF-8b

```
1
GGCTGGGCTAGGAGCCGCCGCCTCCCTCCCGCCCAGCGATGTATTCAGCGCCCTCCGCCTGCACTTGCCTGTGTTTACAC
                                                MetTyrSerAlaProSerAlaCysThrCysLeuCysLeuHis
81
TTCCTGCTGCTGTGCTTCCAGGTACAGGTGCTGGTTGCCGAGGAGAACGTGGACTTCCGCATCCACGTGGAGAACCAGAC
PheLeuLeuLeuCysPheGlnValGlnValLeuValAlaGluGluAsnValAspPheArgIleHisValGluAsnGlnTh
161
GCGGGCTCGGGACGATGTGAGCCGTAAGCAGCTGCGGCTGTACCAGCTCTACAGCCGGACCAGTGGGAAACACATCCAGG
rArgAlaArgAspAspValSerArgLysGlnLeuArgLeuTyrGlnLeuTyrSerArgThrSerGlyLysHisIleGlnV
241
TCCTGGGCCGCAGGATCAGTGCCCGCGGCGAGGATGGGGACAAGTATGCCCAGCTCCTAGTGGAGACAGACACCTTCGGT
alLeuGlyArgArgIleSerAlaArgGlyGluAspGlyAspLysTyrAlaGlnLeuLeuValGluThrAspThrPheGly
321
AGTCAAGTCCGGATCAAGGGCAAGGAGACGGAATTCTACCTGTGCATGAACCGCAAAGGCAAGCTCGTGGGGAAGCCCGA
SerGlnValArgIleLysGlyLysGluThrGluPheTyrLeuCysMetAsnArgLysGlyLysLeuValGlyLysProAs
401
TGGCACCAGCAAGGAGTGTGTGTTCATCGAGAAGGTTCTGGAGAACAACTACACGGCCCTGATGTCGGCTAAGTACTCCG
pGlyThrSerLysGluCysValPheIleGluLysValLeuGluAsnAsnTyrThrAlaLeuMetSerAlaLysTyrSerG
481
GCTGGTACGTGGGCTTCACCAAGAAGGGGCGGCCGCGGAAGGGCCCCAAGACCCGGGAGAACCAGCAGGACGTGCATTTC
lyTrpTyrValGlyPheThrLysLysGlyArgProArgLysGlyProLysThrArgGluAsnGlnGlnAspValHisPhe
561
ATGAAGCGCTACCCCAAGGGGCAGCCGGAGCTTCAGAAGCCCTTCAAGTACACGACGGTGACCAAGAGGTCCCGTCGGAT
MetLysArgTyrProLysGlyGlnProGluLeuGlnLysProPheLysTyrThrThrValThrLysArgSerArgArgIl
641
CCGGCCCACACACCCTGCCTAGGCCACCCCGCCGCGGCCCCTCAGGTCGCCCTGGCCACACTCACACTCCCAGAAAACTG
eArgProThrHisProAla
721
CATCAGAGGAATATTTTTACATGAAAAATAAGGAAGAAGCTCTATTTTTGNACATTGNGTTTAAAAGAAGACAAAAACTG
801
AACCAAAACTCTTGGGGGGAGGGGTGATAAGGA
```

Fig. 3B

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, 5/10, C07K 14/50, 14/515

A3

(11) International Publication Number: **WO 00/05369**

(43) International Publication Date: 3 February 2000 (03.02.00)

(21) International Application Number: PCT/US99/12839

(22) International Filing Date: 20 July 1999 (20.07.99)

(30) Priority Data:

60/093,397	20 July 1998 (20.07.98)	US
09/150,684	10 September 1998 (10.09.98)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	60/093,397 (CIP)
Filed on	20 July 1998 (20.07.98)
US	09/150,684 (CIP)
Filed on	10 September 1998 (10.09.98)

(71) Applicant *(for all designated States except US)*: CURAGEN CORPORATION [US/US]; 11th floor, 555 Long Wharf Drive, New Haven, CT 06511 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SHIMKETS, Richard, A. [US/US]; 191 Leete Street, West Haven, CT 06516 (US).

(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P. C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
15 June 2000 (15.06.00)

(54) Title: NUCLEOTIDE SEQUENCES AND AMINO ACID SEQUENCES OF SECRETED PROTEINS INVOLVED IN ANGIOGENESIS

(57) Abstract

Novel angiogenesis/anti–angiogenesis secreted proteins and the nucleic acid sequences which encode them are disclosed by the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 99/12839

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N5/10 C07K14/50 C07K14/515

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 99 40193 A (ZYMOGENETICS INC) 12 August 1999 (1999-08-12) abstract seq. ID 2 of WO9940193 (ZAP03) is identical to seq.ID 2 of present application claims 1-24 ---	1-7
P,X	WO 99 15653 A (FERRARA NAPOLEONE ;GENENTECH INC (US); SCHWALL RALPH (US); BOTSTEI) 1 April 1999 (1999-04-01) abstract Seq. ID 4 of WO9945653 is identical to seq. ID 2 of present application. claims 1-47 --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2000	04.04.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 99/12839

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KIM I. ET AL.: "Molecular cloning and characterization of a novel angiopoietin family protein, angiopoietin-3" FEBS LETTERS, vol. 443, 29 January 1999 (1999-01-29), pages 353-356, XP002126378 the whole document	1-7
X	PRÖLS F. ET AL.: "Assignment of themicrovascular endothelial differentiation gene 1 (MDG1) to human chromosome band 14q24.2->q24.3 by fluorescence in situ hybridization." CYTOGENET. CELL GENET., vol. 79, 1997, pages 149-150, XP002133850 the whole document	8-14
X	DATABASE EMBL [Online] Accession No. P97554, 1 May 1997 (1997-05-01) PRÖLS F. ET AL.: "rat microvascular endotelial differentiation gene 1 (MDG1)" XP002133852 the whole document	8-14
P,X	DATABASE GENBANK [Online] Accession No. AF083247, 21 June 1999 (1999-06-21) YE M. ET AL.: "Homo sapiens MDG1" XP002133853 the whole document	8-14
X	OHBAYASHI N. ET AL.: "Structure and function of the mRNA encoding a novel fibroblast growth factor, FGF-18." J. BIOL. CHEM., vol. 273, no. 29, 17 July 1998 (1998-07-17), pages 18161-18164, XP002133851 the whole document	15-27
X	WO 98 16644 A (ZYMOGENETICS INC) 23 April 1998 (1998-04-23) abstract examples 1-12 seq. IDs 1,2	15-27

-/--

3

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 99/12839

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVIS S ET AL: "Isolation of Angiopoietin-1, a ligand for Tie2 receptor, by secretion-trap expression cloning" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 87, no. 87, page 1161-1169-1167 XP002102730 ISSN: 0092-8674 the whole document ---	1-27
A	MAISONPIERRE P C ET AL: "ANGIOPOIETIN-2, A NATURAL ANTAGONIST FOR TIE2 THAT DISRUPTS IN VIVO ANGIOGENESIS" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 277, page 55-60 XP002046280 ISSN: 0036-8075 the whole document ---	1-27
A	BECK L JR AND D'AMORE P A: "Vascular development: cellular and molecular regulation" FASEB JOURNAL,US,FED. OF AMERICAN SOC. FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD, vol. 11, no. 11, page 365-373-373 XP002102631 ISSN: 0892-6638 the whole document -----	1-27

INTERNATIONAL SEARCH REPORT

In. .ational application No.
PCT/US 99/12839

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/12839

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1-7) - complete

   Isolated polynucleotide and corresponding polypeptide of angiopoietin-3 (seq. IDs 1,2).

   Corresponding vectors, recombinant host cells, production processes.

2. Claims: (8-14) - complete

   Idem as subject matter 1, but limited to hMEDG1 (Seq. IDs 3,4)

3. Claims: (15-27) - complete

   Idem as subject matter 1, but limited to FGF-8b (Seq. IDs 5-8)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ıal Application No
PCT/US 99/12839

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9940193 A	12-08-1999	AU 2493699 A	23-08-1999
WO 9915653 A	01-04-1999	US 5972338 A	26-10-1999
		AU 9388098 A	12-04-1999
WO 9816644 A	23-04-1998	AU 4758397 A	11-05-1998
		BR 9712348 A	31-08-1999
		CZ 9901315 A	17-11-1999
		EP 0931148 A	28-07-1999
		NO 991796 A	16-06-1999
		PL 332851 A	25-10-1999
		US 5989866 A	23-11-1999